Cyanotoxins: sampling, sample processing and toxin uptake

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Cyanotoxins

The presentation will concentrate on the following commonly occurring cyanotoxins: hepatotoxic microcystins/nodularins, cytotoxic cylindrospermopsin and neurotoxic anatoxin-a. In addition, there are several other cyanotoxins that deserve attention in local or national monitoring programmes: anatoxin-a(S), saxitoxin family, dermatotoxic alkaloids, lipopolysaccharides, BMAA etc.

Matrices; where, what, when and how to sample

Cyanotoxins are known to occur in a number of matrices and sample types: a) Water – source/recreational waters (fresh, estuarine, brackish & marine) and treated water. b) Biological materials: phytoplankton including food supplements, zooplankton, shellfish, fish, terrestrial animals, sea birds, aquatic plants, agri- and horticultural products etc. The main sources of exposure from the human health point of view are drinking water, recreational waters, shellfish and fish, and possibly for some consumers, food supplements. The role of crop plants as a source of exposure is unclear. Comprehensive monitoring of lakes and reservoirs (which may have both water abstraction and recreational use) requires extensive resources as the sampling should have coverage in temporal, horizontal and depth dimensions. Foodstuffs should be monitored at least during bloom periods of cyanobacteria and immediately after blooms.

Sample clean-up for microcystins, anatoxin-a and cylindrospermopsin

Most cyanotoxins can be extracted with (acidified) aqueous methanol. Microcystins and nodularins are usually concentrated on reversed-phase solid-phase extraction cartridges (SPE; C18 or polymeric materials) which have limited clean-up capacity. For microcystins in raw and treated waters, there is an ISO standard in development. Immunoaffinity cartridges, now commercially available, can offer superior clean-up for the peptide toxins in difficult matrices. Anatoxin-a can be concentrated on either C18 (after pH adjustment to 9.6) or on weak cation exchange sorbents. Cylindrospermopsin is very hydrophilic and can be concentrated on graphite carbon SPE columns. Simultaneous SPE of several cyanotoxin classes has been a challenge due to the large differences in analyte polarity and structure. We suggest the use of polymeric mixed-mode materials with both reversed-phase and cation-exchange functionalities for the simultaneous SPE of microcystins, nodularins, anatoxin-a and cylindrospermopsin (e.g. Waters Oasis MCX).

Matrix effects

Various matrix components and co-extracted substances make the analyte clean-up, identification and quantitation difficult. These interfering substances can be of either organic (e.g. humic substances, proteins and other biological macromolecules) or inorganic (for microcystins e.g. chlorine, Fe^{3+}, Al^{3+}) nature. In LC-MS both suppression and enhancement of the analyte signal are possible.

Toxin accumulation in shellfish and fish

Shellfish are readily contaminated by many cyanotoxins and other phycotoxins. Fish flesh is usually safe for consumption (there has been some exceptions) but stomach/intestinal content and internal organs, especially the liver, can contain considerable amounts of cyanotoxins.

Toxin accumulation in plants

Toxin accumulation in plants could either reduce crops or cause health problems for plant consumers. The best documented cyanotoxin group in this context is microcystins which are known to accumulate in a number of terrestrial and aquatic plants. Microcystins can either enter the plants through roots or remain on (in) the leaves after spray irrigation with toxin-containing water.